CLINICAL STUDY

Mycotic VulvoVaginitis: Epidemiology, Pathogenesis and Profile of Antifungal Agents

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Abstract

Objective

VulvoVaginitis is the most common gynecological problem affecting millions of women world wide. At some point in their life time, nearly 75% of all women experience an attack of *Candida* Vulvovaginitis. Little is known about the prevalence of different causes of Vulvovaginitis and risk factors for this entity in Saudi Arabia. This survey was conducted to study the etiologic agents associated with mycotic Vulvovaginitis and review some predisposing factors correlated with this type of infection in Al -Madina Al- Munawarah, Saudi Arabia.

Methods

High vaginal swabs (HVS) specimens were collected from 1000 patients attending gynecological out patients clinics of two general hospitals in Al-Madinah Al-Munawarah . Specimens were cultured on specific medium for yeasts and identification of the positive isolates was carried out using "API" kit. Antifungal sensitivity pattern of the isolates was tested out using "Candifast" kit. Proteolytic enzyme activity of the isolates was also detected.

Results

Three hundred and forty nine positive cases of yeast infection out of 1000 diagnosed cases representing 34.9% were recorded in this study. These positive cases were classified on the basis of the risk factor as; diabetic (28.9%); pregnant, (32.1%); pregnant and diabetic (7.5%); Menopause (5.5%); oral contraceptive users (10.6%); post hysterectomy (1.4%) and no observed factor in 14% of the cases. Twenty one different species belonging to 7 genera were recovered in this study. The genus *Candida* was the most common (93.4%) and included 14 different species. *Candida albicans* was the highest in prevalence (51.3%). The non-Candida species were *Saccharomyces cervisiae* (2.9%); *Rhodotorula rubra* (1.1%); *R. minuta* (0.9%) *Debaryomyces hansenii* (0.6%); *Trichosporon mucoides* (0.6%); *Cryptococcus neoformans* (0.3%) and *Pichia ohmeri* (0.3%). Most of the *Candida* species were sensitive towards nystatin, amphoterian B and fluconazole and resistant to other azole drugs. *Saccharomyces cervisiae* and *Debaryomyces hansenii* were sensitive to almost all the tested antifungal drugs.The rest of the species were variable in their pattern. Proteolytic activity of *C. albicans* reached its maximum value after 72 hours. Neutral proteases was the highest at pH 6.5 followed by alkaline proteases at pH 8.2 and the least amount was acid protease at pH 3.5.

Conclusion

The results of this survey which is the first study done in this region threw some light on the prevalence, etiology, sensitivity profile of the etiologic agents and the risk factors of Vulvovaginitis in Al-Madina Al-Munawarah Saudi Arabia. Awareness towards the increase incidence of Vulvovaginits needs more attention to be paid for fungal infection and antifungal sensitivity.

Key words: Fungal infection, Vulvovaginitis , epidemiology , antifungal , enzyme activity

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Introduction

VulvoVaginitis is a general term used to describe infection or inflammation of the Vulva and vagina. Inflammation of vagina due to infectious agents is very common, both as an overgrowth of normal or common colonizers, or as a frank infection. The most common causes of Vulvovaginitis are yeast, bacteria, protozoa, viruses and parasites.[1]. Egan[2] had reported that Vulvovaginitis is the most frequently gynecological diagnosis encountered by physicians who provide primary care to women.

There are number of vaginal candidiasis risk factors have been identified. The increased susceptibility of women to develop vaginal candidiasis is known to be correlated with the high levels of reproductive hormones and increase in the glycogen content in the vaginal environment. The incidence in the overall population is found to be increased significantly during pregnancy; in those using high dose estrogen contraceptive pills, after an antibacterial treatment regime, and was also more frequently reported in diabetic women [3].

Vulvovaginitis caused by Candida species represents 20-30% of the overall infection. It is a common cause of morbidity, in women. Some women have infrequent occasional episodes of varying severity that respond to antifungal treatment, while others suffer from recurrent, often chronic candidiasis [4]. *Candida albicans* was the most common type of yeast infection (91.8%). Odds[5] had found that *C. glabrata* to be common yeast other than *C. albicans* to be isolated. Certain yeast species are commonly associated with antifungal resistance. Resistance to amphotericin B has been demonstrated in *Candida lusitaniae* [6], other *Candida spp*. Such as, *C.guilliermondii C. inconspicua, C. kefyr, C. krusei, C. rugosa*[7], and *Trichosporon sp.*[8]. Additionally, azole (e.g fluconazole) resistance has been demonstrated repeatedly in *C.glabrata and C.krusei* [9].

The invasion of host tissues by microbial cells possesses constitutive or inducible hydrolytic enzymes which destroy or degrade constituents of cell membranes leading to membrane dysfunction and or physical disruption. Since host cell membranes are made up from lipids and proteins, it is obvious that these biochemical processes include the largest part of enzyme attack[10].

The production of hydrolytic enzymes, especially secreted aspartic proteinases, as key virulence to determinants has been comprehensively studied [11]. Proteinase production by *C. albicans* is associated with pathogenicity[12],[13] Proteinase enzyme is located as a mannoprotein, functions as a ligand for attachment to host cells and was identified as virulence determinant[14].

Materials and Methods

Selection of Subjects

One thousand patients attending gynecological outpatients clinics of two general hospitals (Ohod and the Maternity hospitals in Al-Madinah Al-Munawarah),complaining of vaginal discharge and itching, over a period of 8 months (October ,2007 to May ,2008) were tested for the presence of Vulvovaginal fungal infection.

Collection of the clinical samples

This study protocol was approved by the Deanship of Scientific Research of Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia.

High vaginal swabs (HVS) specimens were taken from each patients, Each HVS was cultured on a Sabouraud dextrose agar (SDA) plate (supplemented with 500 mg/liter of chloramphenicol .The plates were incubated at 37 C for 5 to 7 days before discarding as negative. Only patients who yielded heavy growth of yeasts in culture were selected for the study. Other Bacterial, Parasitic infections were not tested in this study.

The following tests were carried out for each culture[15].

- Purification of the culture.
- Yeast morphology on corn & rice-meal tween 80 agar media
- Germ tube test

API 20 C AUX for identification of the yeast isolate

Identification of the isolates was carried out using API 20 CUX kit (Bio Merieux, SA Marcy- L' Etoile, France)[16].

The pathogenic potentialities of the isolates were tested for

- Production of proteolytic enzymes.
- Production of lipolytic enzymes
- And Blood Haemolysis: [17]

Sensitivity of the isolated strains to some antifungal agents using "Candifast ES Twin Kit".

Antifungal sensitivity testing of pathogenic *Candida* species were carried out using "CANDIFAST ES Twin kit" (ELITECH, France SAS)[18]. It provides a sensitivity profile against 7 antifungal agents (amphotericin B, nystatin, flucytosine, econazole, ketoconazole, miconazole and fluconazole).

Quantitative determination of proteolytic enzymes activity[19,20].

Enzyme assay

0.1 M sodium citrate buffer containing 2 gm BSA/litre and the pH adjusted at 3.5, 6.5 and 8.5.

- Culture supernatant, assay medium were kept on ice.
- Reaction starts by adding 0.1 ml supernatant + 0.9 ml assay medium.
- Rapidly shaking at 37°C for 10 min.
- Add equal volume of 5% trichloroacetic acid.
- After and additional 10 min, the reaction mixture was centrifuged.
- The supernatant was decanted, and the absorbance at 280 nm was read against blank containing distilled water.

Enzyme units are expressed as the amount of tyrosine in micromoles released per minute per milliliter of culture supernatant (10^8 cells of *Candida* species).

At the same time of enzyme assay, the growth rate was determined (absorbance at 660 nm).

Results

Out of 1000 female patients (ranged from 16 to 65 years) attending the out patients gynecological clinics with Vulvovaginal itching and discharge, *Candida* species and other non- *Candida* were isolated from 349 out of the 1000 tested case. Positive cases represented 34.9% (**Table 1**). Positive cases were classified according to the suspected predisposing factor for fungal infection: 101 diabetic (28.9%), 112 pregnant (32.1%), 26 pregnant and diabetic (7.5%), 19 Menopause (5.5%), 37 oral contraceptive (10.6%), 5 post-hysterectomy (1.4%) and 49 with no observed factors (14%) as shown in **Table 2.**

Patients were classified into 5 groups according to their age. The maximum positive cases were recorded in the second group (age range 26-35) followed by the first group (age range 15-25) shown in **Table 3.**

Data of **Table 6** revealed that 21 yeast species were identified belonging to 7 genera; *Candida, Cryptococcus, Debaryomyces, Pichia, Rhodotorula, Saccharomyces* and *trichospron*.

The prevalence of non-Candida species was 6.6% (23/349) (**Table 6**). The non-Candida isolates included; *Saccharomyces cervisiae* 10 (2.9%); *Rhodotorula rubra* 4 (1.1%) *Rhodotonula minuta* 3 (0.9%), 2 case of each: *Debaryomyces hansenii,* and *Trichosporon mucoides* each representing 0.6% and one case of each of *Cryptococcus neoformans* and *pichia ohmeri* (representing 0.3% in **Table 6**).

In this study, the genus *Candida* recorded the highest prevalence 326 caes representing 93.4% of the total positive. *Candida albicans* was the most prevalent species recovered in this study. It was isolated from 179 case constituting 51.3% of the positive cases. *Candida glabrata* came next in rank; it was isolated from 78 case constituting 22.3% of the positive cases. *Candida lusitaniae, C. tropicalis C. pseudotrepicalis and C. krusei* were of low occurrence representing 5.4%, 4.3%, 3.4% and 2.6 respectively. The rest of the isolated *Candida* spp. were of rare occurrence (**Table 6**).

Candida albicans recorded the highest isolation rate in all studied groups. It was recovered as follows: 54 cases out of 101 in diabetic group, 62 out of 112 in the pregnant group, 14 out of 26 of the diabetic - pregnant group; 9 out 19 in Menopause group, 21 out of 37 in oral contraceptive group, 2 out of 5 in post hysterectomy group and 17 out of 49 in the unobserved factor group (**Table 5**).

The tested species proves their ability to hydrolyze casein and fat which indicted their potentiality and their implication in the pathogenesis process. Also almost all (except *Saccharomyces* and *Debaryomyces spp.*) isolates were positive in the blood haemolysis test which proves their invasive and disseminated form.

Table 1: Prevalence rate of the test sample

Samples	Total rested samples	Positive cases	Percentage %
Patients	1000	349	34.9

Table 2: Distribution of yeast positive cases according to suspected predisposing factors

Predisposing Factor	No. of Cases	Percentage %
Diabetic	101	28.9
Pregnant	112	32.1
Pregnant and Diabetic	26	7.5
Menopause	19	5.5
Oral contraceptive	37	10.6
Post-hysterectomy	5	1.4
No observed factor	63	14
Total	349	100

Table 3: Distribution of yeast positive cases according to patient's age

Groups of patients	No. of cases	Percentage %
15-25	118	33.8
26-35	135	38.7
36-45	64	18.3
46-55	28	8.1
56-65	4	1.4
Total	**349**	**100**

Table 4: Frequency and percentage of isolated genera and species of yeasts from test sample

General and species of yeast	No. of species	No. of cases	%
Candida	14	326	
(1) C. albicans	-	179	51.3
(2) C. glabrata	-	78	22.3
(3) C. lusitaniae	-	19	5.4
(4) C. tropicalis	-	15	4.3
(5) C. pseudotropicalis	-	12	3.4
(6) C. Krusei	-	9	2.6
(7) C. famata	-	4	1.1
(8) C. guilliermondii	-	3	0.9
(9) C. parapsilosis	-	2	0.6
(10) C. ciferii	-	1	0.3
(11) C. dubliniensis	-	1	0.3
(12) C. pelliculosa	-	1	0.3
(13) C. rugosa	-	1	0.3
(14) C. zylanoides	-	1	0.3
Saccaromyces	1		
(15) S. cervisiae	-	10	2.8
Rhodutorula	2		
(16) R. ruburu	-	4	1.1
(17) R. minuta		3	0.9
Debaryomyces	1		
(18) D. hansenii		1	0.6
Trichosporon	1		0.6
(19) T. mucoides		2	
Cryptococcus	1		
(20) C. neoformans	-	1	0.3
Pichia	1		
(21) P. ohmeri	-	1	0.3
Total	**21**	**349**	**100**

Table 5 : Distribution of the isolated yeast species among the different groups of patients

Groups of patients		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total	
																							No.	%
Diabetic		54	26	4	3	5	-	1	-	-	-	1	1	-	-	3	2	-	1	-	-	-	101	28.9
Pregnant		62	21	8	6	3	2	1	-	-	1	-	-	1	-	1	2	1	1	-	-	-	112	32.1
Diabetic & pregnant		14	5	-	2	-	3	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	26	7.5
Menopause		9	3	1	1	-	-	1	-	-	-	-	-	-	-	1	1	1	-	1	-	-	19	5.5
Oral contraceptive		21	8	2	-	-	2	1	-	-	-	-	-	-	-	2	1	1	-	-	-	-	37	10.6
Post hysterectomy		2	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	1	-	5	1.4
No observed factor		18	15	15	1	4	2	-	2	2	-	-	-	-	1	-	-	-	-	-	-	1	49	14
Total	Total	179	78	19	15	12	9	4	3	2	1	1	1	1	1	10	4	3	2	2	1	1	349	100
	%	51.3	22.3	5.4	4.3	3.4	2.6	1.1	0.9	0.6	0.3	0.3	0.3	0.3	0.3	2.8	1.1	0.9	0.6	0.6	0.3	0.3		

Table 6: Percentage of Sensitivity and Resistance of the isolated pathogenic yeast towards the common antifungals

Organism	Total Count	AB%		NY%		FCT%		ECZ%		KTZ%		MCZ%		FCZ%	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
C. albicans	179	67.8	32.2	82	18	78	22	78.5	21.5	70.6	29.4	49.7	50.3	53.7	46.3
C. glabrata	78	64.1	35.4	84.6	15.4	62.8	37.2	65.4	34.6	52.6	47.4	30.8	69.2	37.2	62.8
C. tropicalis	19	84.2	15.8	89.5	10.5	100	0	89.5	10.5	73.7	26.3	47.4	52.6	31.6	68.4
C. lusitaniae	15	66.7	33.3	66.7	33.3	80	20	66.7	33.3	80	20	53.3	46.7	46.7	53.3
C. pseudotropicalis	12	75	25	91.7	8.3	50	50	50	50	58.3	41.7	41.7	58.3	83.3	16.7
C. Krusei	12	33.3	66.7	83.3	16.7	25	75	33.3	66.7	33.3	66.7	16.7	83.3	58.3	41.7
C. famata	4	25	75	50	50	50	50	25	75	25	75	25	75	25	75
C. guilliermondii	3	0	100	66.7	33.3	66.7	33.3	33.3	66.7	33.3	66.7	33.3	66.7	33.3	66.7
C. Parapsilosis	2	0	100	100	0	0	100	0	100	0	100	0	100	100	0
C.Ciferii	1	100	0	100	0	100	0	10	0	100	0	100	0	100	0
C. dubiliensis	1	100	0	100	0	100	0	0	100	0	100	0	100	0	100
C. pelliculosa	1	100	0	100	0	100	0	100	0	100	0	100	0	0	100
C. rugosa	1	100	0	100	0	100	0	100	0	100	0	100	0	100	0
C. Zylanoides		100	0	100	0	100	0	0	100	0	100	0	100	0	100
Saccharomyces cerevisiae	10	80	20	90	10	90	10	80	20	80	20	70	30	70	30
Rhodotorula rubra	4	75	25	25	75	50	50	50	50	50	50	75	25	75	25
R. minuta	3	66.7	33.3	66.7	33.3	66.7	33.3	66.7	33.3	66.7	33.3	66.7	33.3	66.7	33.3
Debaryomyces hansenii	2	100	0	100	0	100	0	100	0	100	0	50	50	100	0
Trichosporon mucoides	2	50	50	50	50	50	50	25	75	50	50	0	100	75	25
Pichia ohmari	1	100	0	100	0	100	0	0	100	0	100	0	100	100	0
Cryptococcus neoformaus	1	100	0	100	0	100	0	100	0	100	0	0	100	100	0

Anti fungal sensitivity testing of the isolated species

Sensitivity testing of the isolated species was carried out using candi fast kit. It provides a sensitivity profile against 7 anti fungal agents (Ampoteriin B, Nystatin, 5-flurocytosine, Econazole, Ketonazole, Miconazole and Fluconazole).

The test showed that most *Candida* isolates were sensitive to Nystatin. Most of the isolates of *Candida albicans* (80%), the most common species in this investigation was also sensitive to Nystatin, Amphotericin B and some Azole compounds especially Fluconzole .

Almost all the isolates of *Saccharomyces cervisiae* and the two isolates of *Debaryomyces hansenii* were sensitive to all antifungal drugs (**Table 6**).

Data in Table 6 showed the variable response of the other isolated species towards the tested antifungal drugs used in this study.

Production of extracellular proteinases by *Candida albicans*

In this study the determination of the acid, neutral and alkaline proteases activities was carried out at the following pH values (3.5, 6.5 and 8.5). The growth rate and enzyme activity were determined every 12 hours for a total incubation time of 96 hours. **Table 7** and **Figure 1** showed that the proteolytic activity reach its maximum values after 72 hours. This result was relevant to maximum growth rate of the tested strain, C. *albicans.* The proteolytic activity of *C. albicans* that measured at pH 3.5 reached its maximum value of 685 µmol/ml. Also the proteolytic activity of C. *albicans* reach to the value of 643 µmol/ml at pH 8.2. From the above results, it was found that the neutral proteases (pH 6.5) were released in appreciable amounts in C. *ablicans* followed by alkaline proteases (pH 8.2) while the least amount recorded was of acid proteases (pH 3.5) **Table 8.**

Table 7: Protease enzyme productivity at different incubation periods by C. albicans at pH value 3.5, 6.5 and 8.2

Inc. Period / pH value	12h	24h	36h	48h	60h	72h	84h	96h
	Protease enzyme productivity (µmol) tyrosine/ml							
pH 3.5	335	395	383	427	461	493	345	409
pH 6.5	455	543	528	595	608	685	486	592
pH 8.2	575	758	478	557	580	643	413	535

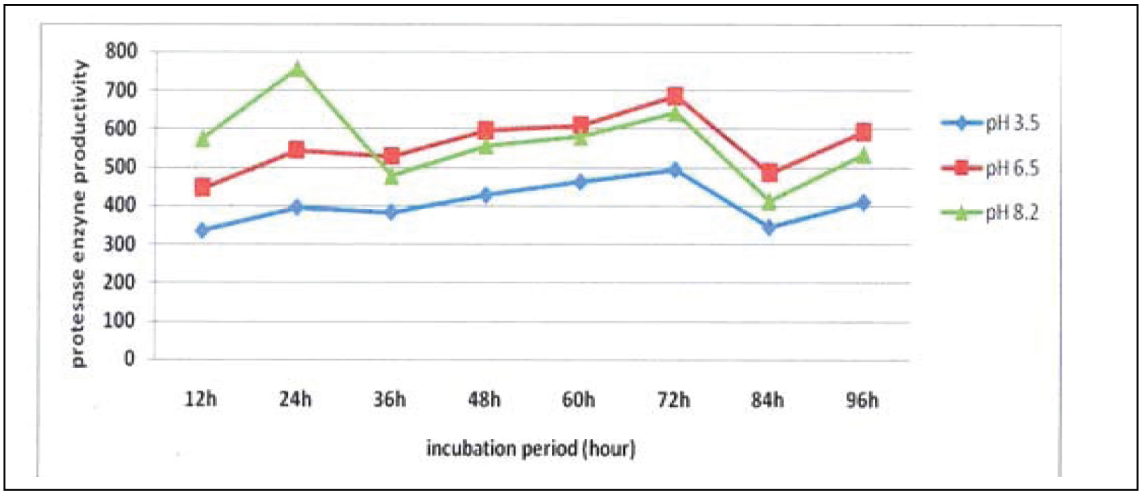

Figure1: *Protease enzyme productivity at different incubation periods by C. albicans at pH values 3.5, 6.5 and 8.2*

Table 8: Protease standard values using different tyrosine concentrations at pH values 3.5, 6.5 and 8.2

pH value Tyrosine conc.	O.D.		
	pH3.5	pH 6.5	pH 8.2
0.1	0.032	0.033	0.026
0.3	0.056	0.065	0.036
0.5	0.070	0.079	0.062
0.7	0.087	0.086	0.087
1.0	0.118	0.108	0.098
2.1	0.178	0.166	0.178

Discussion

Over the last several decades, medical advances have become available that make human life more safe. Factors such as transplant surgery and concomitant immunosuppressive therapies, anti-cancer therapies, medical devices that traverse the protective skin barrier (e.g. central venous lines, catheters, etc.), broad spectrum antibacterial therapies, corticosteroid therapies, certain disease states (e.g. malignancy, human immunodeficiency virus infection, etc.), and others have contributed to increased numbers of immunocompromised individuals. These immune-deficient individuals are at higher risk for yeast infections and the spectrum of offending species is ever increasing. Species that were considered to be saprophytic are becoming opportunists causing human diseases[21].

In the present study, 349 (34.9%) positive cases were recovered out of 1000 tested patients.

This result is in agreement with that of Margriti[22] , who reported that mycotic Vulvo vaginitis is the most common clinical manifestation of fungal infections causing human mycoses; the incidence occurs in 10% of women, while, during pregnancy the incidence achieves 30% of cases. *Candida* species was the most common pathogen in 35.5% of symptomatic women and 15% of asymptomatic controls[23] . Data of this study revealed that yeast vaginal infection among the classified groups of patients was; pregnant (32.1%) diabetic (28.9%), oral contraceptive users (10.6%), pregnant and diabetic (7.5%), menopause (3.4%), post-hysterectomy 1.4% and (14.1%) with no observed factors.

It is well documented that pregnancy and diabetes mellitus increases the rate of vaginal colonization and infection with *Candida*[24,22,25]. Potential risk factors for Vulvovaginal candidiasis have been identified, including women of child bearing age, post menopausal women who have underlying risk factors such as hormone replacement therapy or immunosuppression caused by medications or diseases , using high estrogen containing combined oral contraceptive pills, vaginal douching, some sexual behaviors, contraception devices, (diaphragm, intrauterine device etc.) and antibiotics [26,27,28,25] .Concerning the etiology in the present study, the genus *Candida* (represented with 14 species) was the most common etiologic agent documented by culture, microscopy and the results of the API Kit. This genus constituted 93.4% of the total positive cases, while the prevalence of the non-Candida genera was 6.6% of the total positive cases. This result is similar to that of Saporiti *et al*[29]. *Candida albicans* was by far the most common pathogen detected in this study (51.3% of the positive cases). Vasquez *et al*.[30] reported that, the major opportunistic pathogen has been *Candida albicans*. The proportion of genital *C. albicans* in symptomatic women ranges from approximately 90% in Australian samples31 to approximately 65% in Belgium[32], Turkey[33] and Saudi Arabia[34]. The non-albicans *Candida* species isolated during this study represented 42.1% including *C. glabrata* (22.3%), *C. tropiclis* (5.4%), *C. lusitaniae*

(4.3%), *C. pseudotropiclis* (3.4%), C. *Krusei* (2.6%), *C. famata* (1.1%), *C. guilliermondii (0.9%), C. parapsilosis* (0.6%) and the following species *C. ciferii, C. dubliniensis, C. pelliculosa, C. rugosa* and *C. zylanoides* were of rare occurrence and each represented 0.3% of the total positive cases. This study confirmed that *Candida glabrata* currently ranks second as causative agent of vaginal candidal infection and are common in immunocompromised persons or those with diabetes mellitus as reported by Geiger et al[28] and Paul et al[14] Two specialized clinics had reported rates of 10% to 20% of non-albicans Vulvovaginal candidiasis, and *Candida glabrata* had consistently been the dominant species [27, 35]. It is worth mentioning that there is a variation in the yield of the species of *Candida*, where 14 species were recovered belonging to this genus, some of them may be reported as causal agent of Vulvovaginitis rarely or for the first time. *Saccharomyces cervisive* (2.9%); *Rhodotorula rubra* and *minuta* (2%); each of *Debaryomyces hansenii* and *Trichosporon mucoides* represented (0.6%) and 0.3% for each of *Cryptococcus neoformans* and *Pichia ohmeri*. This list was also reported as causal agents of vaginitis by many authors[2,36,37] while other species were recorded as causal agents of other clinical cases [38,39] . It was surprising to report one case caused by *Cryptococcus neoformans*. Cryptococcal infection is opportunistic and occurs most commonly in immunocompromised patients. Cryptococcal infection usually presents as meningoencephalitis or pulmonary infection. Skin, bone and genital infections are very rare [41]. Cryptococcal infection of the vagina was reported before by Chen et al.[42] and Ranganathan et al.[43]. The case recorded in the present study is one of the rare cases to report cryptococcal vaginitis. Drug resistance is a major problem in treating yeast infections[44]. At present, yeast infections are usually treated as a general fungal infection and agents such as the polyene, amphotericin B or the newer azole drugs, which are intended to control a broad array of fungi, are used[45]. The *Candida* species collected during this study with high frequency rate (*C.albicans*, 179 strain and *C.glabrata*, 78 strain),gave meaningful data with the sensitivity test. The overall of *C.allbicans* isolates showed resistance to "AB (32.2%); NY (18%); FCT (22%); ECZ (21.5%); KTZ (29.4%); MCZ (50.3%) and FCZ (46.3%). While *C. glabrata* showed the following resistance pattern "AB (35.4%); NY (15.4%); FCT (37.2%); ECZ (34.6%); KTZ (47.4%); MCZ (69.2%) and FCZ (62.8%). Other collected species with low or rare frequency gave meaningless data with the antifungal sensitivity test. Generally the incidence of amphotericin B- resistant *candida* species in our study was 35.5%; Nystatin 17.5% Fluctosine 27.9%; Econazole, 29.4%; ketokonazalo 36.5%; Miconazole, 57.4% and Fluconazole, 51.2%. This incidence of antifungal resistant *candida* species in our results is higher than that reported by other studies [45,46,47,48]. Sanglard and Odds [50] concluded that *Candida albicans* and related species pathogenic to man become more resistant to antifungal agents, in particular triazole compounds, by expression of effux pumps that reduce drug accumulation, alteration of membrane sterol composition resistance towards most of the tested antifungal drugs. In the case of Vulvovaginal candidiasis, an analysis of clinical isolates indicates that resistance is due to not only to resistant strains of *C. albicans* but also to an increasing number of non-*albicans Candida* strains. Various *Candida* species appear to develop resistance to the commonly used drugs at frequencies much higher than that for *C. albicans* [50]. In the present study, the *Candida* species other than *albicans* and *glabrata* showed variable patterns of resistance and sensitivity and most of them were sensitive to Nystatin except some isolates of *C. krusei* and *C. guilliermondii.* Mashburn and Facum[52] suggested that uncomplicated vaginal candidiasis is easily treated with topical azole antifungal medications in single or short-term doses. This class of drugs is usually more effective than the older nystatin class of drugs. Capoor *et al.*[46] reported that the spectrum of candidiasis has changed with the emergence of non-candida species and acquired antifungal resistance. Other non-Candida species such

as *Saccharomyces cervisiae* and *Debaryomyces hansenii* were sensitive to most of the tested antifungal drugs. While *Rhodotorula rubra, Rh. Minuta Trichosporon mucoides, Pichia* ohmeri and *Cryptococcus* neoformans were variable in their resistance and sensitivity pattern towards the tested antifungal drugs. . Many yeasts and molds are known to secrete extracellular proteases[53] For *Candida* species, some investigators have reported the determination of proteolytic activity[54] whereas others have detected no activity[54]. This discrepancy may be attributed to the conditions used for eliciting and measuring protease activity. In this study, it was found that the level of proteolytic enzyme was low during the earlier exponential growth phase and reached its maximum value after 72 hours. This clearly shows a direct correlation between the growth rate of yeast cells and the proteolytic activities of *Candida* isolates. This correlation was also reported by[56] who connected between the proteolytic production and the growth rate of yeast cells. Many workers agreed that *Candida* species need 72 hours, incubation time for the maximum protease production[57,58]. In this study, the neutral proteases (pH 6.5) were released in appreciable amount; 685 µmol/ml. in case of *C. albicans.* This followed by alkaline proteases 643 µmol/ml. These results coincide with that of Dostal *et al.*(2003) who reported that the pH value was the critical factor in proteolytic activity because relatively small pH shifts can cause changes in extracellular proteolytic activity. Also, these results were supported by Taylor *et al.*[53] who found that the human vaginal infections were accompanied by elevation of pH values (neutral to alkaline) and subsequently the protease activity reached to the maximum levels. Odds[5] and Fidel *et al.*[59] reported that *Candida albicans* produces a higher amount of proteases in comparison with other *Candida* species.

Conclusion

The incidence of fungal infection and vulvo vaginal candidiasis is increasing rapidly, in relation to the growing number of diabetic patients and immunocompromised individuals in the population.

Candida albicans is the most common cause of Mycotic Vulvovaginitis . Other non-albicans species such as *Candida glabrata* and *tropicalis* , which are increasing in frequency are also involved . Topical and oral antifungal therapies are effective. Resistance of the Candida spp. towards the commonly used antifungal drugs is increasing.

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